Immunobiology 218 (2013) 755–761

Contents lists available at SciVerse ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Review

Ancestral gene and "complementary" antibody dominate early ontogeny☆

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ARTICLE INFO

Article history:
Received 5 May 2012
Accepted 24 August 2012

Keywords:
Ancestral forces
Complementary antibody
INIM
Ovarian physiology

ABSTRACT

According to *N.K. Jerne* the somatic generation of immune recognition occurs in conjunction with germ cell evolution and precedes the formation of the zygote, *i.e.* operates before clonal selection. We propose that it is based on interspecies inherent, ancestral forces maintaining the lineage. Murine oogenesis may be offered as a model. So in C57BL/10BL sera an anti-A reactive, mercapto-ethanol sensitive glycoprotein of up to now unknown cellular origin, but exhibiting immunoglobulin M character, presents itself "complementary" to a syngeneic epitope, which encoded by histocompatibility gene A or meanwhile accepted ancestor of the ABO gene family, arises predominantly in ovarian tissue and was detected statistically significant exclusively in polar glycolipids. Reports either on loss, pronounced expressions or *de novo* appearances of A-type structures in various conditions of accelerated growth like germ cell evolution, wound healing, inflammation and tumor proliferation in man and ABO related animals might show the dynamics of ancestral functions guarantying stem cell fidelity in maturation and tissue renewal processes. Procedures *vice versa* generating pluripotent stem cells for therapeutical reasons may indicate, that any artificially started growth should somehow pass through the germ line from the beginning, where according to growing knowledge exclusively the oocyte's genome provides a completely channeling ancestral information. In predatory animals such as the modern-day sea anemone, ancestral proteins, particularly those of the p53 gene family govern the reproduction processes, and are active up to the current mammalian female germ line. Lectins, providing the dual function of growth promotion and defense in higher plants, are suggested to represent the evolutionary precursors of the mammalian immunoglobulin M molecules, or protein moiety implying the greatest functional diversity in nature. And apart from any established mammalian genetic tree, a common vetch like *Vicia cracca*, may represent an ancient model of protected reproduction mirroring A-reactive "complementarity" already in a plant. The in its seeds developed, and from the number of chromosomes depending amount of an anti-A_1 specific glycoprotein suggests promotion of germination while simultaneously exerting protection from a soil bacterium, which intriguingly is immobilized by human anti-A immunoglobulin as well. Moreover, in a mammalian ovary the lectin of *Dolichos biflorus* detects again histo (blood) group A-determining N-acetyl-D-galactosamine epitopes, here signalizing activity of embryonic stem cells. So apparently based on identical, ancestral structures, the dual function of growth promotion and defense, predetermined in a plant genome, might be preserved right up to dominate early mammalian ontogeny.

An evolutionary genetic act, preceding the formation of the zygote, or operating before clonal selection, was decades ago proposed by *N. K. Jerne*: "*I assume that antibodies directed against self-components on cell surfaces have some important functions in ontogeny, namely in cell to cell recognition that is needed for the formation of specialized tissues and for morphogenesis. Cell recognition, which must be fundamental in even the most primitive metazoan, e.g. sponges may require the presence of histocompatibility antigens and of complementary "antibody" molecules at cell surfaces. The present hypothesis proposes that the germ cells of an animal carry a set of v-genes determining the combining sites of antibodies directed against a complete set of a certain class of histocompatibility antigens of the species to which this animal belongs. The theory explains how a functional immune system can develop through a selection pressure exerted by self-antigens, starting during a period in early ontogeny that precedes clonal selection by foreign antigens*" (Jerne 1971).

Histocompatibility genes and v-genes for the respective "complementary" antibody structures of a species arise in the tissues of its members in parallel. This at first mysteriously appearing

Abbreviation: INIM, innate immunity.

☆ The cited experiments of the author were performed at Gastroenterology Research Laboratory, Department of Medicine, University of Iowa College of Medicine, Iowa City, Iowa, USA and Research Laboratories Chemie Grünenthal GmbH, 52062 Aachen, Germany.

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0171-2985/$ – see front matter © 2012 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.imbio.2012.08.277

antithetic cooperation was supposed to represent the crucial evolutionary driving force in tissue maturation and morphogenesis, while an explanation might be provided by the already dual functions of pluripotent plant glycoproteins or lectins. So the cow vetch *Vicia cracca*, for instance may exert an ancient model of protected reproduction or INIM involving A-like structures. The in its seeds developed anti-A_1-reactive glycoprotein apparently promotes their growth, but simultaneously protects them from a soil bacterium, which is immobilized by human anti-A immunoglobulin as well (Jones 1964). And as in humans (Cambi and Figdor 2003) also in the plant discrimination between self and not self may occur by different carbohydrate compositions of cell surfaces. Primarily considering these functions, lectins are discussed to be the evolutionary precursors of the immunoglobulin M molecule, while the lectin-mediated pathway of complement activation might precede that of the immunoglobulin pathway in evolution (Vasta et al. 1999). Moreover, the lectin of *Dolichos biflorus*, which in a mammalian ovary detects A-determining N-acetyl-D-galactosamine epitopes (Arend and Nijssen, 1977b) signalizes the activity of embryonic stem cells (Nash et al. 2007) and demonstrates again the preservation of in phylogenesis approved structures and functions. So in early mammalian ontogeny, which will be considered to start in oogenesis, particularly tailored epitopes on germ cell surfaces should mirror receptors also for "complementary" glycoproteins, which are produced not in the course of an adaptive immunological response, but in parallel, either at random or genetically programmed.

An example for the expanded concept, proposing interspecies specific, ancestral forces dominating germ cell evolution may be given by the "complementary" binding of the murine "natural" or today "innate" anti-A reactive immunoglobulin M from C57BL/10 infertilized females to a syngeneic epitope arising in parallel predominantly in ovarian tissue (Arend and Nijssen 1976) and suggests encoding by ancestral power. The ovary, through its role in reproduction, is designed as an immunologically privileged organ. In the genital ridge of the early fetal period "complementary" allelic activities of germ cells and of somatic cells may be brought into contact for the future concert of endocrinological and immunological actions. They might be provided in a particularly tailored composition of immunocompetent cells and complement functions (Spanel-Borowski 2011). And the first steps of INIM channeled by ancestral forces maintaining the lineage in diploid organisms, may here be observed in a physiological microcosm on its own, where the encoded A-type epitope primarily displays the ancestor's authentic power.

The beginning in the understanding of "self" and "not self" and their final biological alliance should be connected to the discovery of the interspecies specific ABO blood group antigen or histocompatibility system (Landsteiner 1901) which acquired significance far beyond the original goal in transplantation medicine, and apart from its position in anthropology (Smith 1960; Saitou and Yamamoto 1997; Watkins 1966) meanwhile leads into molecular paleontology (Eisler 1930; Morgan 1942, 1944; Morgan and Schutze 1943; Turcot-Dubois et al. 2007). So investigations on the ABO carbohydrates and the analysis of the respective antibody specificities (Wiener 1941, 1942) including their functional relevance opened the gate into the current knowledge of genetics, normal and pathological cell membrane physiology in man and animals and into the structural relationships between eukaryotic and prokaryotic cells and their various interactions in biology (Morgan 1942; Morgan and King 1943; Morgan and van Heynigen 1944; Watkins and Morgan 1962; Hakomori 1985, 1999; Yamamoto et al. 1990; Yamamoto 2004). Studies on the long-term evolution of the CAZY glycosyltransferase 6 ABO gene family demonstrate the great genetical ABO polymorphism involving the variety of synthesizing and modifying enzymes which appear to dominate the phylogeny from fishes up to man (Turcot-Dubois et al. 2007). Tissues are now known to express these antigens always in a tissue intrinsic way. For each epithelial tissue, antigenic expression is related to the stages of cell maturation from the germinal layer up to surface epithelium (Oriol et al. 1992). Qualitatively and quantitatively changing expressions of epitopes demonstrate the different embryonal stages in ontogeny which, one has to imagine always starts from only a single cell.

The close biological relation of the system to the environment soon became evident. Fractions of the human ABO isoagglutinins are stimulated to some extent by antigenic structures namely from gram negative bacteria (Springer et al. 1961; Springer and Horton 1969), while discrepant reactions to environmental stimulations already suggested alternative origins of isoagglutinin production, *i.e.* blood group O people only responded to increased enteral absorptions (Arend and Fehlhaber 1969). So with respect to sex dependent distributions of murine anti-A production and the analogy to the lectins of *Vicia cracca* and its different sets of chromosomes, growth processes for the first time were discussed (Arend 1971). On the other side, the ABO and Lewis group epitopes themselves were discovered as pathogen receptors (Huang et al. 2003; Fumagalli et al. 2009), which are specifically reactive with various viral and bacterial antigens and, moreover so are supposed to be engaged in the evolution of the ABO polymorphism (Seymour et al. 2004).

The first rules of ABO blood group inheritance were developed by Von Dungern and Hirszfeld (1910), who already established the serological paternity exclusion, while only in 1924 Felix Bernstein, cited by Crow (1993), detected the correct inheritance pattern of multiple alleles at one locus. When routes to a common molecular ancestor from the ABO evolutionary tree were constructed, considering the development of primate genes and their homologous genes, type A appeared to be identified as the common ancestral gene for the ABO hominoid and Old World monkey blood groups, while three B alleles evolved independently on the human, gorilla, and baboon lineages (Saitou and Yamamoto 1997). The investigations are supported by cloning of rat genes, which again suggested that the ancestral gene may be of "A"-type, because all rat A-sequences reported so far possess all exons compared with human and the other primates (Turcot et al. 2003).

But apart from any constructed genetic trees, the great polymorphism and dynamics of the human type "A" arising in a variety of phenotypes or subgroups (Hakomori 1985, 1999) may already reflect its dominant position in the ancestry.

To the best of our knowledge phylogenetical trees were up to now not constructed from the in various strains and phenotypes existing mouse. Nonetheless its genome contains the human AB gene equivalent and apparently has derived from the same ancestral gene (Yamamoto et al. 2001). Unlike the human A and B genes which encode two distinct glycosyltransferases with different donor nucleotide-sugar specificities, the murine AB gene only occurs as an equivalent of the human *cis*-AB, which prevails in mouse populations and encodes both, A- and B-transferase activities. But the published immunohistochemical pictures obtained with commercial murine monoclonal anti-A and anti-B antibodies and submaxillary gland tissues from a C57BL and an ICR strain of mice (Yamamoto et al. 2001) display a marked expression only of A. So a structural dominance of a functional A over the genetically associated B might here again signalize its special ancestral position also in the mouse, which will provide the model for the widened perception of somatic generation of immune recognition and INIM.

The ovary of the C57BL/10 inbred mouse in particular, where the female displays a normal estrous cycle and fertility, was discovered as the fortunate experimental tool and may serve as a magnifier for a biological event occurring in non-reproductive tissues probably below the level of detection. Human "natural" anti-A isoantibody

Fig. 1. Exclusive inhibition of murine anti-A antibody-mediated lysis of human blood group A_1 erythrocytes by syngeneic ovarian polar glycolipids from 80 day old C57BL10/J unfertilized females. (a) Control (phosphate-buffered saline); polar glycolipids; (b) ovary; (c) testis; (d) liver of female; (e) liver of male; (f) stomach of female; (g) stomach of male; (h) salivary gland of female; (i) salivary gland of male; (j) spleen of female; (k) spleen of male; (l) kidney of female; (m) spleen of male; (n) brain of female; (o) brain of male; (p) thymus of female; (q) thymus of male; (r) heart of female; (s) heart of male. Methods according to Arend and Nijssen (1977a).

here detects type A reactivity in entodermal tissues such as male and female liver and stomach as well as in the gonads (Arend 1979), while the animal itself produces a mercaptoethanol-sensitive anti-A reactive glycoprotein (Arend 1971) appearing predominantly in unfertilized females, binds significantly to syngeneic ovarian glycosphingolipids, which developed growth-dependently in parallel up to peak levels at puberty (Fig. 1). These water-soluble ovarian structures (Arend and Nijssen 1977a, 1977b) (Fig. 2) exhibit an epitope which is characterized by N-acetyl-D-galactosamine in terminal linkage as detected by reactions with the lectins of *Dolichos biflorus* and *Helix pomatia* (Arend and Nijssen 1977b).

So in the C57BL/10 female mouse the association of autoreactive ovarian with xeno-reactive structures in non-reproductive entodermal tissues particularly presents the great polymorphism of the common histo-(blood)-group antigen A in a single inbred organism (Arend 1979) and the opportunity for experimental and theoretical approaches to the mechanisms of germline encoded innate immunity called INIM, while this immunobiological event might invisibly occur in the other murine strains as well, because as already cited above, the AB gene prevails in all of them (Yamamoto et al. 2001).

Ovariectomy performed at the age of 20 days causes rapid disappearance of the murine anti-A reactivity from the sera and demonstrates the crucial role of the ovary in this antibody or glycoprotein production, which apart from the promoting function of the X chromosomes (Libert et al. 2010) might involve a variety of factors. First of all, the removal of the organ eliminates the estrogen mediated B-cell activity (Holmdahl et al. 1989) and to an unknown extent diminishes antibody levels. Moreover, it does not only take away the site of epitope appearance, but probably that of the early antibody production as well. Because the abruptness of the effect of ovariectomy and the pattern of disappearance (Fig. 3) accounts for its early production starting in the ovary, from which the first bulk of the autoreactive immunoglobulin appearing in the circulation will be secreted. In fact, ovarian production of immunoglobulins, in particular against auto-reactive epitopes arising *de novo* in maturation must today be considered to be established (Hoek et al. 1997), while the ovarian A-reactive epitope discovered, might neither represent a tissue specific ovarian, nor strain specific murine structure. As a common mammalien one, and even more, it occurs widespread in nature, and its pronounced appearance in the murine C57BL/10 ovarian maturation merely signals over-expression, used as a fortunate experimental tool.

At first glance the formation of the anti-A-reactive mercaptoethanol-sensitive immunoglobulin may look as an

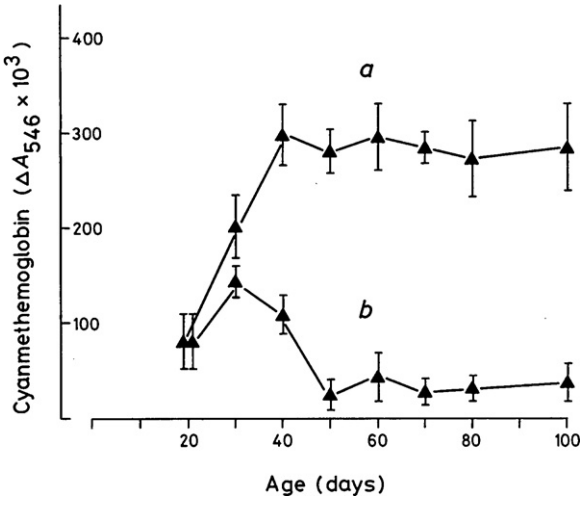

Fig. 2. Effect of early ovariectomy performed at the age of 20 days on the development of "natural" anti-A hemolysin production: (a) sham-operated animals; (b) ovariectomized animals. Each value represents the arithmetic mean and standard deviation of the lytic activities determined in 10 single sera against human blood group A_1 erythrocyte.
From Arend and Nijssen (1977a).

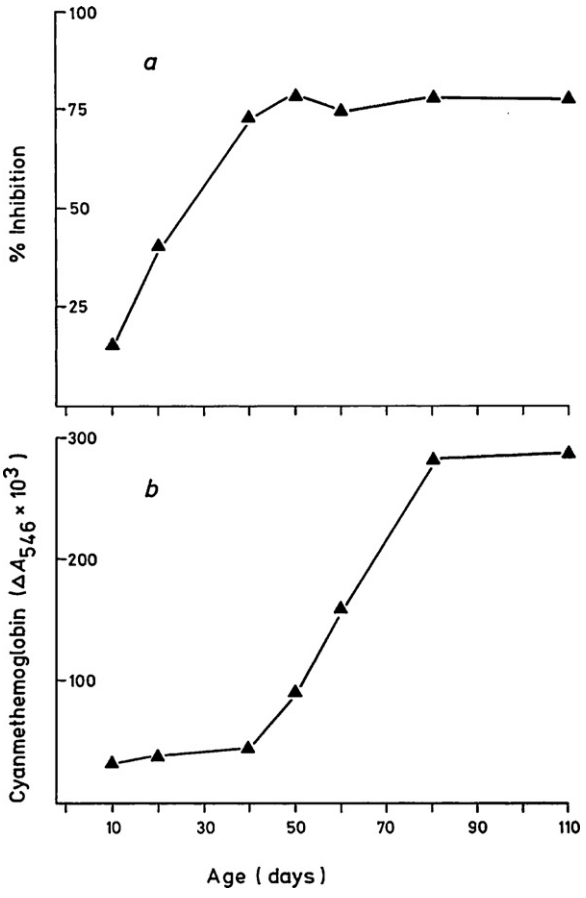

Fig. 3. Growth-dependent formation of ancestral A-specific ovarian epitope and "complementary" antibody up to puberty. (a) Age-dependent development of receptor activity for syngeneic anti-A hemolysin in C57BL/10 murine ovaries. The inhibitory activities are given as % inhibition exerted by 10 μg ovarian glycolipid on the lytic power of 20 μl pooled serum from 80-day-old C57BL/10 females against human blood group A_1 erythrocytes. (b) Age-dependent appearance of anti-A hemolysin activities in the sera of C57BL/10 female mice. Each value represents the activity measured in 20 μl pooled sera against human blood group A_1 erythrocytes.
From Arend and Nijssen (1977a).

adaptive, primary immune response, and up to now it was respectively discussed (Arend and Nijssen 1977a, 1977b), although never associated or followed by detectable 7S or IgG levels (Arend 1971), and despite a secondary response further missing throughout the observations (data not shown). It hereby equates the property of the human "natural" or innate anti-A immunoglobulin M, because in human sera, apart from conditions after adverse transfusions or AB incompatible gravidity, anti-A reactive IgG immunoglobulins have up to now been described only for blood group O sera (Rieben et al. 1992), where however any anti-AB cross-reactivity, signalizing for instance, an adaptive response to environmental, microbial structures (Arend 1971), was not sufficiently excluded. And phenotype independent anti-A as anti-B reactive immunoglobulin G, purified by affinity chromatography, was detected exclusively in the micromolar range with A- and B specific trisaccharides (Spalter et al. 1999), while in other investigations, performed again on pooled sera, epitope specificity profiling on ABO glycoconjugates showed such autoreactivity to be unrelated to the "classic" ABO(H) blood group structures (Obukhova et al. 2012): The IgG-autoantibody directed against blood group A or B disaccharides was found without consideration to the presence of fucose, but requiring the absence of elongating sugar X in composition of the Gal(NAc)α1-3(Fucα1-2) Galβ1-X terminated carbohydrate chain, while alloanti-AB antibodies demanded a minimum of the trisaccharide Gal(NAc)α1-3(Fucα1-2) Gal epitope and recognized the elongated type specific tetrasaccharides. They appeared to be a small set of specific yet cross-reactive antibodies which detect all backbone types of A or B antigens, rather than being a collection of specific antibodies, each of which discovers a different type of A or B antigen.

On the other hand, there are several explanations, for an isolated, or persisting primary immune response, as, for instance, the type of antigen and genetic background of the responding organism (Rudbach and Reed 1997), and depending from the topography, antigen application may meet upon different distributions of immuno-competent or antibody secreting B cells (Sell et al. 1970).

However, not even an isolated primary, adaptive response as source of the murine anti-A production could be suggested. In fact, the kinetics of epitope and early antibody together up to puberty (Fig. 4), as well as probably topographically associated production account much more for a parallel encoding of two opposite, or "complementary", distinct allelic functions. These findings might again be in accordance with the picture of the growth-dependent innate anti-A specific immunoglobulin M production in humans, where over more than a century no evidence for any adaptive A-reactive immune response could be provided. Moreover, as again to the mouse, anti-A cross-reactivities originating from environmental antigenic stimulations by lipopolysaccharides of *Escherichia coli O86*, were clearly unmasked by absorption experiments, separating the "adaptive" from "innate" anti-A antibody (Fig. 4).

Beyond those finally open questions a maturing ovary always has to be considered as kind of an organism on its own, involving an intrinsic immunobiology. One has to imagine that it may generate a complete offspring's genome not only in phylogenetically lower species, but even in man, where parthenogenetical potentials are still alive (Kim et al. 2007; De Fried et al. 2008). The cellular traffic in oogenesis involving uncountable intercellular correlations will be dominated by those between stem cell populations and oocytes, first of all their potentials to be transformable in each other (Virant-Klun et al. 2008, 2011; White et al. 2012), moreover, to differentiate into any somatic tissue intrinsic cell (Pacchiarotti et al. 2010; Loewer et al. 2010). So progenitor cells harvested from bovine follicles become endothelial cells (Merkwitz et al. 2010). But cellular profiling in immunohistochemical and/or radiohistological experiments are required to elucidate the kinetics of the cell lineages providing the synthesis of the "A-type"

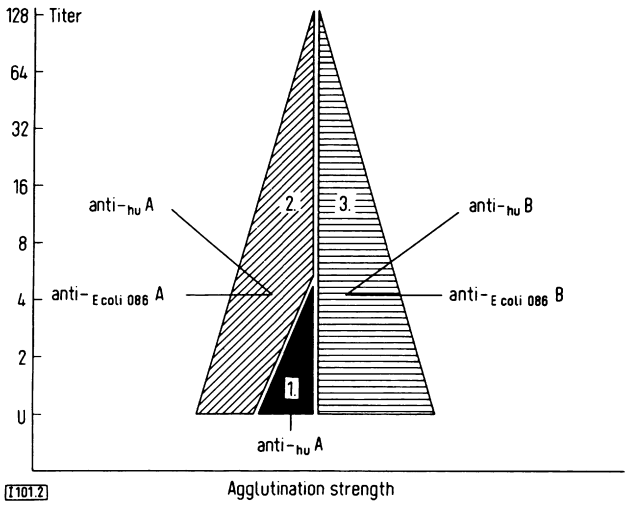

Fig. 4. Separation of innate from adaptive anti-A antibody in C57BL/10J female. Composition of C57BL/10 female immune sera, scheme constructed from absorption and neutralization experiments: 1 = Innate, mercaptoethanol sensitive anti-A specific immunoglobulin, exclusively reactive with or absorbable by human blood group A erythrocytes and non-reactive with lipopolysaccaride of *Escherichia coli* O86. 2 = Adaptive anti-A immunoglobulin, induced by and absorbable with *E. coli* O86 lipopolysaccharide and cross-reactive with human blood group A and B erythrocytes. 3 = Adaptive anti-B immunoglobulin, induced by *E. coli* O86 lipopolysaccharide and cross-reactive with human blood group A and B erythrocytes.
From Arend (1971).

epitope and presentation in the ovary, hypothetically in molecular conjunction with its "complementary" immunoglobulin (Fig. 3). It has to be taken into account, that immunoglobulins were already discovered in chicken eggs (Yamamoto et al. 1975) and an IgM like glycoprotein in the oocytes of salmons (Yousif et al. 1995). Although these proteins are up to now considered to be derived from the maternal circulation, germ cells of an animal, carrying a set of v-genes (Jerne 1971) may be involved in the formation of cytokeratin-positive cells as potential dendritic cells, regulating "*Footmarks of Innate Immunity in the Ovary* -" (Spanel-Borowski 2011) and come to maturity under the power of "natural antibody" (Bavry et al. 2004). So in early ontogeny the obvious activity of an ancestral gene like A in particular, unmasked by its encoded specific epitope in conjunction with the respective "complementary" antibody should not be surprising. The ovary therefore may function as an immunologically privileged organ, designed to control in cooperation between immunological and endocrinological actions the physiologically cyclic inflammations which arise consistently with ovulations and follicular ruptures respectively, and inevitably releases various toxic as well as provocatively autoreactive structures. Consequently it is forced to organize its defense systems by itself: Complement cascades and complement danger signals, autophagocytosis of damaged or dying cells, as production of proteins and peptides sustaining the repair, and/or maintaining the normal ovarian function have to be organized. And cytokeratin positive cells, again might act as potential dendritric cells and play the basic role in this particular tissue intrinsic homeostasis (Spanel-Borowski 2011). It necessarily involves the proposed growth-promoting encoding of an auto-reactive ancestral epitope generated in conjunction with its "complementary" antibody molecule, but not restricted to the ovary, where under the fortunate experimental conditions of the C57BL/10 female anatomy and physiology it just becomes visible. In the course of growth and maturation or tissue renewal, affording reprogramming of somatic stem cells into pluripotency, it invisibly should occur in any other tissue as well. The respective enzymatic potentialities have already been detected in tissues predominantly of the entodermal layer (Miyazaki et al. 1987).

The histo-"incompatible" growth dependent A-specific ovarian structures of the C57BL/10 inbred mouse reflect activity of an "aberrant" glycosyltransferase arising somewhere in the course of early germ cell evolution, which divides as rearranges the genetic material and activates silent or "cryptic" genes, either by haploidization (Kocabas et al. 2006), or by reprogramming developmental events particularly in parthenogenesis (Edwards 2007; Gao et al. 2007). The X-chromosome exhibits its crucial evolutionary power, because considering exclusively the parthenogenetic haploid blastocysts, alone those containing the X-chromosome are reported to survive (Tarkowski 1977). Inbreeding apparently promotes parthenogensis (Uyenoyama 1985) which involves creation of pluripotent, respectively embryonic stem cells (Isaev et al. 2001; Kim et al. 2007; De Fried et al. 2008), might generate foreign genoms, involving the revival of an ancestral gene and reappearance of its functional epitope, like "A".

Therefore parthenogenetic potentials arising here probably besides normal ovulations, might even more explain the close topographical relation of the epitope which may meet the antibody secreting B-cells, arising in parallel from embryonic stem cells located in the ovary. The first bulk of the antibody appearing in the circulation may indeed be secreted from the ovaries themselves, while on the way to maturation the lymphatic system in non-reproductive tissues will more and more take part in its production. Further, the carbohydrate changes when passing through the germ line, and in the course of maturation arises in new, tissue intrinsic expressions. So in non-reproductive growing tissues of the adult C57BL/10 mouse carbohydrates loose auto reactivity, instead develop xenoreactive properties in entodermal organs (Arend 1979). As already mentioned, for each tissue, antigenic expression is related to the stages of cell maturation from the germinal layer up to surface epithelium (Oriol et al. 1992): The original ancestral genetic function, which in the murine ovary primarily encoded a somehow complete human-like A-epitope on the surface of pluripotent embryonic stem cells, may further channel the germline and in conjunction with "complementary" immunoglobulins modulate the tissues on the way to maturation. The descending somatic cells have been specialized to their tissue intrinsic structures, and like immunoglobulins already C-type lectins here discriminate between self and not self by different carbohydrate composition on cell surfaces (Cambi and Figdor 2003). Dependent from the type of tissue respective numbers of cells always keep the original, complete ancestral information and so as appropriate to be reprogrammable to pluripotency, which inconsistently becomes evident in various conditions of accelerated growth.

Consequently, an already tissue-specifically developed cell, which simply would artificially be forced to restore or display the completely original germ cell epitope, is expected not to survive. And experiments originally designed for another question indeed fulfilled this very expectation: Using lentiviral gene transduction, human blood group A antigen expression was induced on mouse cells and one group of animals additionally sensitized by human blood group A_1 erythrocytes (Xiaohu et al. 2010). In fact, tissue damage associated with deposition of antibody and complement as macrophage infiltrations was not restricted to sensitized animals, but occurred less pronounced in non-sensitized animals as well. Because as hypothetically expected, the surfaces of modulated hepatocytes certainly had developed topographically inappropriate, *i.e.* "incomplementary" carbohydrate structures, which became complement signalizing and exposed to the auto-destructive, nonetheless channelling processes of "innate immunity", where now the anti-A reactive immunoglobulin M not any longer behaves "complementary".

These observations might show once again that the innate murine anti-A reactive immunoglobulin accommodates widely divergent qualities and physiologic functions, which obviously are exerted highly dependent from the epitope presenting type of cell and/or environment. In the laboratory a destructive power is experimentally exhibited by complement activation after binding to the membrane of human blood group A_1 red cells used as experimental tool. This effect should represent the strongest contrast to its proposed growth promoting or sustaining involvement in syngeneic ovarian maturation, where the old term "antibody" could almost be misleading and probably therefore by Jerne himself was set in quotation marks (Jerne 1971). Indeed "natural" or today called "innate" antibodies inducing intracellular signaling are meanwhile considered to be involved in growth control (Wang and Chow 2000), where "naturally" occurring immunoglobulin M molecules apparently display an extremely functional diversity (Brissac et al. 1999). So the destructive power of an immunoglobulin *in vitro* against an erythrocyte surface carrying a specific epitope used as experimental tool, does not say anything about its physiological function *in vivo*. Again, as the phylogenetical follower of plant lectins, the innate immunoglobulin M acquired its polyreactive function of growth stimulation and inhibition and/or cell destruction, while the lectin-mediated pathway of complement activation procedures may precede the immunoglobulin pathway in evolution (Vasta et al. 1999).

The ancestral epitope A which we called "evolutionary" (Arend 2011) appears as a dynamic polymorphic structure being obviously independent from the "classic" phenotype A (Miyazaki et al. 1987) and may somehow be tailored always to the respective requirement probably resolved by up to now uncountable structural options. And produced beyond ontogeny in any condition of accelerated growth in man and animals, phenotype independent pronounced expression or *de vovo* production of A-type structures may signalize activation of "cryptic" functions in reprogrammed somatic stem cells exhibiting poly- or pluripotency. It is known for decades, that the original A and B-reactive phenotypes in tumor tissues are often diminished or lost, while *de novo* expressions of "inappropriate" A-type structures are inconsistently observed in tumors of blood group O (Okada et al. 1987; David et al. 1993) as well as in blood group B people (Hakomori 1999). The A-type *de novo* expression was here defined by monoclonal antibody, which reacts with monofucosyl type 1 chain A (ALe^d), but not by the antibody, directed to difucosyl type 1 chain A (ALe^b), mono- or difucosyl type 2 chain A, or type 3 or type 4 chain A. And because the monoclonal antibody reactivity was abolished by α-*N*-acetylgalactosaminidase, the findings identified this specific expression of "inappropriate" A with the structure monofucosyl type 1 chain A (ALe^d) (Clausen et al. 1986; Metoki et al. 1989). Moreover, *de novo* A-specific or -like structures were also detected in tumor tissues of mice, either spontaneously (Hirota et al. 1992a) or in response to chemical or pharmacological treatment (Hirota et al. 1992b). But further cellular profiling, particularly in human cancer, should elucidate to what extent the A-type *de novo* expressions within a diseased tissue imply normal somatic stem cells as well, which might be reprogrammed to pluripotency in hypothetically reactive self-renewal processes. Because in benign disorders, such as wounded human skin, an expression of histo-blood group A type 3 antigen was suggested to be involved especially in stabilization of inflammation (Nosaka et al. 2008), while on the other side loss of A-like structures in wound healing (Dabelsteen and Fejerskov 1974) may again show the dynamics of an ancestral epitope. So the position of the A-gene in the ancestry is far more mirrored in changing expressions as well as in functions in physiology (Engelmann et al. 1992; Chehrehasa et al. 2008; Arend 2011).

However, the authentic significance of any ancestral genetic power may finally consist in channeling cell differentiation and maintaining the lineage. This view comes up following the intriguing reports on the p63/p73 common ancestor of the much

older p53 gene family. It is found in almost all invertebrates and preserved its structural properties for over one billion years of evolution (Lane et al. 2011; commented by Levine 2012). Further, the p53family genes in vertebrates still appear to maintain their primordial functions in germ line surveillance and reproduction, and accomplish the critical role of the ancestral p53pathway of genes in protecting primarily the mammalian female germ line and fertility. The earliest living common ancestor with humans that contain a p53 family gene member is detected already in the evolution of predatory animals and represented in the modern-day diploid sea anemone (Belyi et al. 2010). And to respeak, the A-gene, not yet found in plants but mirrored by *Vicia cracca*, obviously became engaged in haploidizations of murine germ cell maturation, necessarily involving activation of opposite "cryptic" genes and providing the proposed "complementary" allelic forces maintaining the lineage, while apart from evolutionary position, within the ABO family, may act as a functional backbone of growth processes *per se* (Arend, 2011).

So the distributions of the in parallel arising "complementary" polyfunctional glycoprotein, or early immunoglobulin M, in male and female C57BL/10 mice (Arend 1971; Arend and Nijssen, 1976; Arend and Nijssen, 1977a), might be in accordance with the critical role of the p53 pathway of genes and illustrate the predominance of the female germ line and its preferred protection in younger ontogeny.

In view of the growing knowledge in stem cell physiology uncountable investigations to construct pluripotent and tissue specific stem cells for therapeutical applications have already been undertaken and discussed (González et al. 2011), while possible requirements of early germ line activities like ancestral information, which obviously channel cell differentiation and tissue maturation were up to now not considered. Meanwhile a long history of failures demonstrate, that new cell types or genomes created simply by gene transduction, forced expression of specific genes (Xiaohu et al. 2010), or pure nuclear transfer, may neither function nor survive. Chemical treatment or exposure of cells to cell extracts as mismatch repair may transform stem cells even into cancer stem cells implying imponderable therapeutic implications (Vaish 2007). After all, experiments on primordial germ cells obviously failing on the maintenance of the germ cell lineage (Durcova-Hills et al. 2008) might indicate that any reconstruction of tissue specific cells, or artificially started growth must somehow pass through the germ line from the beginning.

Not surprisingly the exchange of the oocyte's genome with the genome of a somatic cell, followed by generation of pluripotent stem cells (Jullien et al. 2011) could enable the production of tissue specific cells, if the oocyte genome is not removed and the somatic cell genome merely added (Noggle et al. 2011). The resultant triploid cells develop to blastocysts, which differentiate into cell types of all three germinal layers. Though a triploid cell procedure with respect to later clinical applications raises questions (Daley and Sobakk 2011), a groundbreaking result here nonetheless demonstrates, that the female germ line alone, channeled by ancestral codes should provide the proposed driving power in the very early ontogeny as might be mirrored, for instance, in the kinetics of both the C57BL/10 murine A-type epitope and "complementary" antibody up to puberty (Fig. 2). In fact, germ cell evolution and INIM, or protected reproduction, formate a physiological unity dominated by ancestral genetical forces, mirrored as early as in a plant genome.

Acknowledgement

Sincere thanks go to Prof. em. K. Spanel-Borowski, Institute of Anatomy, Leipzig, Germany, for her critique and essential suggestions based on her own work on the ovary.

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